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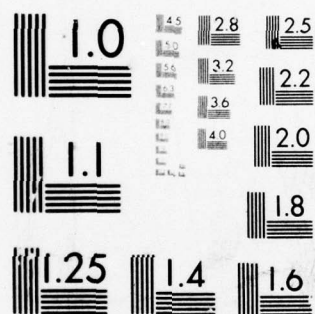
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Dunaeva, T.N. and Shlygina, K.N.

CHANGE IN PHAGOCYTIC ACTIVITY TO TULAREMIA MICROBE IN HIGHLY SENSITIVE
ANIMALS WITH MIXED INFECTION

Zh. M.E.I. 54(4):86-91, 1977

Gamalei Institute of Epidemiology and Microbiology, USSR AMS, Moscow
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It is known that during mixed infections there are observed deviations in the typical development of the illness. We have determined that in types of animals with a low sensitivity to tularemia prior infection by other stimulants activates protective factors and causes an accelerated development of specific immunity reactions. In these cases the animals withstand (? word cut off on Xerox - TRANSLATOR) infection with massive fatal dosages of the tularemia microbe.

In animal types which are highly sensitive to tularemia, in the event of mixed infection there is an increase in the duration of the illness and a decrease in the intensity of septicemia /5,6/. The mechanism of these deviations during tularemia is undetermined (? word cut off on Xerox - TRANSLATOR).

Phagocytosis is a significant factor in the protection of an organism. We studied the absorptive and digestive functions of the neutrophils in rabbits, common field and white mice infected with pseudotuberculosis, and guinea pigs infected with salmonella. The study was carried out in the opsonophagocytary reaction (OPR) with homologous and tularemic microbes, according to the previously described method /7/. The reaction was staged simultaneously

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in animals with various periods of infection and in control (intact) individuals. In each group 4 to 5 animals were studied. In field mice and mice blood was taken from the heart after their mortification; from guinea pigs -- while alive.

TABLE 1 Phagocytic activity of the neutrophils in the blood of field mice infected with pseudo-tuberculosis

День после заражения DAY AFTER INFECTION	ОФР с туляремийным микробом OPR TULAREMIA						ОФР с псевдотуберкулезным микробом OPR PSEUDOTUBERCULOSIS					
	ABSORPTION индекс поглощения INDEX		INDEX RATIO отношение индексов 60 мин: 30 мин 60 MIN: 30 MIN	PHAGOCYTIZING % фагоцитирующих нейтрофилов NEUTROPHILS		NEUTROPHILS	ABS индекс поглощения INDEX		INDEX RATIO отношение индексов 60 мин: 30 мин 60 MIN: 30 MIN	% фагоцитирующих нейтрофилов NEUTROPHILS		
	INDEX			NEUTROPHILS			INDEX			NEUTROPHILS		
	30 MIN	60 MIN		30 MIN	60 MIN		30 MIN	60 MIN		30 MIN	60 MIN	
	MIN	MIN		MIN	MIN		MIN	MIN		MIN	MIN	
4-й	11,5	5,7	0,49	46	23	10	4,2	0,42	40	17		
7-й	12,2	9,2	0,75	49	37	10	6,0	0,60	40	24		
10-й	12,7	6,0	0,48	51	24	8,7	7,2	0,82	35	29		
14-й	9,2	16,5	1,78	37	66	6,2	4,2	0,65	25	17		
Интактные: INTACT:												
control: 4, 10 & 14th day	4,2	14,3	3,54	17	57	4,5	6,0	1,33	18	24		
control by 7th day	6,0	16,0	2,66	24	64	7,7	12,0	1,55	31	48		

On the day of the study of the OPR in animals at various stages of illness from the primary infection, as well as animals of the control group were infected subcutaneously homolaterally with 0.3 ml dilution tularemia culture (strain No. 503), containing 10 microbe cells per 1 ml, which accorded with 1 Dclm.

In field mice infected with pseudotuberculosis with a dose of 100 000 microbe cells, there was an increase in the absorptive and digestive functions of the neutrophils with respect not only to the

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the homologous but to the tularemic microbe (Table 1). This increase is expressed clearly by the 4th to 10th day after infection with the pseudotuberculosis stimulant, and is less perceptible on the 14th day of illness. In the reaction with the pseudotuberculosis bacteria the functional capacity of the leucocytes was retained for the duration of the entire experiment, which is connected with the formation of a specific immunity in the infected field mice. In intact field mice the absorption index of tularemia microbes increases chronographically by 2.66 to 3.54 times and, in the reaction with pseudotuberculosis bacteria, by 1.33 to 1.55 times, which reflects the varying extent of sensitivity to infection on the part of field mice to these bacteria.

In white mice the OPR was studied on the 7th and 11th day after infection with pseudotuberculosis microbes in a dose of 10,000 microbe cells. The absorption indices for tularemia bacteria after 30 minutes equalled 15.5 and 20.5, respectively, and 13.2 in intact mice. After 60 minutes of reaction the indices rose in mice infected with pseudotuberculosis by 1.07 to 1.5 times, and by 2.17 times in intact specimens. In the reaction to the pseudotuberculosis microbe the ratio of indices on the 7th day of infection comprised 1.37, and 0.59 on the 11th day. In intact mice the absorption index increased 1.98 times.

All 78 field mice infected with tularemia microbes on the 4th, 7th, 10th and 14th days after introduction of the pseudotuberculosis bacteria died, the majority -- in the same period as the control, by the 6th to 7th day. 13 field mice died by the 8th to 10th day. However,

with mixed infection there was a decreased intensity of organ insemination, particularly of the blood, which was expressed distinctly in the case of a 4 to 7 day interval between infections (Table 2). In 11 field mice the results of microscopic study of smears from organs were negative, and the presence of tularemia microbes was determined only by seeding on Yemel'yanova's blood agar with penicillin.

TABLE 2. Insemination by tularemia bacteria of organs in dead field mice during mixed infection

Interval between infections, Days	No. of field mice	Presence of tularemia bacteria in smears					
		Spleen		Liver		Blood	
		+	-	+	-	+	-
4	22	17	5	12	10	5	17
7	19	15	4	13	6	7	12
	18	16	2	15	3	13	5
	19	19	-	17	2	15	4
TOTAL	78	11(14.1%)		21(26.7%)		38(48.7%)	
Control	20	20	-	20	-	20	-

Typical for animals highly sensitive to tularemia was a maximum insemination of III - IV scores /9/ observed in the spleen of 70.5% of the field mice, in the liver of 52.2% and in the blood of only 39.7% of individuals. In 20 control field mice insemination of the organs and blood reached III - IV scores in all cases. During mixed infection vacuolated cells -- monocytes -- were found in smears from organs, and neutrophils with phagocyted tularemia bacteria in the blood.

Among the 78 field mice that died, phagocytal cells were found in the spleens of 26% of the individuals, in the livers of 36.3% and in the blood of 32.4%. With intervals between infections of 4, 7, 10 and 14 days, phagocytic cells were found, respectively, in 63.6, 57.6, 38.8 and 36.7% of the dying field mice. During monotularemia, phagocytic cells are found very infrequently, in consequence of their complete destruction and the filling of the entire field of view with tularemia bacteria.

Of 80 mice infected with tularemia bacteria after 3, 5, 7 and 14 days after the introduction of the pseudotuberculosis bacteria 74 died. 38 died within the same time period as the controls -- by the 6th to 7th day, 22 -- by the 8th to 9th day, and 14 mice by the 10th to 12th day. The intensity of insemination with tularemia bacteria was high, and only in 7 mice was the bacteremia evaluated at I-II scores. Phagocytosis of tularemia bacteria was observed in smears from the spleen in 6.8%, in the liver of 17.7% and in the blood of 19.1%, of the 74 individuals that died. During intervals between infections of 3, 5, 7 and 14 days, phagocytosis was observed, respectively, in 35.3, 40.0, 27.7 and 1.3% of the mice. The surviving 7 mice were infected after 20 days with a minimum dose of tularemia bacteria and died by the 7 to 10th day.

The decrease in septicemia and the frequency of phagocytosis manifestation coincided with the period of increased nonspecific digestive activity by the leucocytes in the first 7 days of the development of pseudotubercular infection. Later, the phagocytosis obtained

specificity with respect to the pseudotuberculosis bacteria and had no observable effect on the multiplication of tularemia bacteria in vitro and in the animals' organisms.

As can be seen from Table 3, in guinea pigs infected with *S. typhimurium* in a dose of 500 million microbe cells, the increase in phagocytary activity to the tularemia microbe was traced over a long period of time. Even 54 days after the infection there was observed some bacteriostatic activity of phagocytosis, as compared to the intact individuals.

TABLE 3. Phagocytic activity of neutrophils in the blood of guinea pigs infected with *S. typhimurium*.

DAY AFTER INFECTION День после заражения	OPR TULAREMIA ОФР с тулярийным микробом						OPR SALMONELLA ОФР с сальмонеллами					
	ABSORB. индекс поглощения INDEX		① отношение индексов 60 мин: 30 мин	% фагоцити- рующих нейтрофилов ②			ABSORB. индекс поглощения INDEX		① отношение индексов 60 мин: 30 мин	% фагоцити- рующих нейтрофилов ②		
	30 мин	60 мин		30 мин	60 мин		30 мин	60 мин		30 мин	60 мин	
	MIN	MIN		MIN	MIN		MIN	MIN		MIN	MIN	
3-й	12,0	8,5	0,72	49	34		29,0	27,0	0,94	80	80	
7-й	15,8	5,7	0,35	63	23		29,5	24,0	0,80	74	58	
9-й	17,0	13,5	0,96	68	54		39	35,2	0,80	90	95	
14-й	12,0	7,7	0,66	48	31		38,8	32,6	0,84	90	90	
22-й	12,6	5,5	0,43	34	26		24,4	22,2	0,95	71	70	
33-й	10,5	18,5	1,76	42	60		48	31,5	0,60	98	80	
54-й	7,3	12,3	1,67	30	48		48,6	47,0	0,90	100	100	
Интakтные	6,5	16,2	2,67	25	65		10,6	21,8	2,38	42	76	

① INDEX RATIO
60 MIN: 30 MIN.
② % PHAGOCYTARY
NEUTROPHILS

24 guinea pigs were infected with tularemia bacteria 3, 7, 9, 14 and 22 days following the introduction of *S. typhimurium*. In 12 of them the OPR was investigated on the 5th day following the infection with tularemia bacteria. An increase in the digestive activity of the neutrophils was observed in 8 guinea pigs. The ratio of absorption indices

after 60 and 30 minutes of the reaction fluctuated between 0.47 and 0.93. In 4 guinea pigs the decrease in the number of microbes in the neutrophils was expressed weakly: absorption indices after 60 minutes remained the same or increased to 1.6 times. In the control guinea pigs infected only with tularemia bacteria the absorption indices increased chronographically by 1.8 to 2.4 times.

9 control guinea pigs died from tularemia by the 8th to 13th day, by the 11th, 6th day on the average. Of 24 guinea pigs infected with mixed infection 19 died. 7 guinea pigs died by the 10th to 13th day, 4 on the 14th to 15th and 8 died within the period 17th to 25th day. The average period before death in the various groups fluctuated between 13.6 and 16.3 days. In some of the guinea pigs who died by the 17th to 25th day antibodies were found in the blood for the tularemia microbe in titers of 1:10, 1:40 and 1:160. In guinea pigs that died by the 13th to 14th day such antibodies were not discovered.

Undoubtedly, the prolonged course of tularemia during mixed infection is connected with the increase in phagocytosis. This is seen clearly when comparing the periods before death of 63 guinea pigs infected with tularemia bacteria in various periods after salmonellosis and listeriosis, and also of the control animals with OPR indicators studied on the day of infection with tularemia (Table 4).

The data concerning the guinea pigs that survived are of interest, too. In previous experiments there were observed cases

of guinea pigs ill with tularemia in concert with other infections -- salmonellosis, pseudotuberculosis and listeriosis. In them were found antibodies against the tularemia microbe and a long-term (up to 40 - 118 days) carrying of tularemia bacteria /6/.

TABLE 4. Comparison of death times for guinea pigs with the digestive activity of neutrophils.

Period of death, Days	Number of Guinea pigs	Absorption index ratio 60 minutes:30 minutes		
		less than 1	1	greater than 1
4 - 11	22	21	1	0
12- 16	26	8	4	14
17- 25	15	2	1	12

In the present experiment, the 5 surviving guinea pigs were examined on the 32nd day following the introduction of the tularemia bacteria, respectively on the 35th - 46th day after salmonella infection. In all guinea pigs there was salmonellosis -- agglutination reaction positive in serum diluted 1:160 to 1:2560. When staging the agglutination reaction with tularemia diagnosis and separate study of lymph nodes, spleen, liver and lungs with the use of bioprobes in white mice, tularemia disease was not found.

In 3 guinea pigs prior to infection an OPR was made which found intensive digestion of the tularemia microbe. Absorption indices during 30 minute exposure equalled 16, 19 and 12, and after 60 minutes these decreased to 7, 8 and 8. The index ratio equalled 0.43, 0.42 and 0.66.

The number of bacteria absorbed by 25 neutrophils, in the first smears, comprised 86, 115 and 51, and in the second ones decreased to 21, 28 and 31. The increase in the digestive capacity of the leucocytes could cause a destruction of a small number of inoculated bacteria (tularemia) in the adaptation phase, which prevented the development of infection. Similar cases during mixed infection were observed infrequently in the previous experiments /5, 6/ as well. Far more frequently there is a description of cases of survival by white mice and guinea pigs infected with plague microbes in conjunction with other microbes /1, 2, 4, 8, 10/.

Bacteriological and serological investigation of the surviving individuals found no plague illness. Probably in these cases, too, the "cross phagocytosis" /3, 8/ had a mortifying effect on the plague microbe during its adaptation period in the organism. Often such animals are considered "unreceptive", "resistant" or insensitive to infection. In fact, in these cases there takes place an insignificant rise in the reception threshold in consequence of temporary activation of the nonspecific protective factor of phagocytosis. Repeated infection of these animals causes the development of fatal infection.

CONCLUSIONS

1. The infection of animals highly sensitive to tularemia with sublethal doses of pseudotuberculosis and salmonella bacteria causes an increase in the absorptive and digestive activity of the

leucocytes to the homologous and tularemia microbes.

2. The activation of the nonspecific protective factor of phagocytosis causes deviations in the typical course of tularemia, causing an increase in the course of the illness, a decrease in the intensity of septicemia and, rarely, an increased sensitivity threshold connected with the death of small doses of the microbe during the adaptation stage.

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CHANGE OF PHAGOCYTIC ACTIVITY TO TULAREMIA MICROBE IN HIGHLY SENSITIVE ANIMALS WITH MIXED INFECTION

T. N. Dunaeva, K. N. Shlygina

→ An increase of the ingestive and digestive capacity of neutrophils to the homologous causative agent and tularemia microbe was revealed by the opsonophagocytic test in *Microtus arvalis*, albino mice and guinea pigs infected with sublethal *Yersinia pseudotuberculosis* and *Salmonella typhimurium* doses. In subsequent tularemia infection some of the animals displayed a reduction of the septicemia intensity, prolongation of the disease and elevation of the susceptibility threshold. Period of manifestation of the inhibitory action on tularemia coincide with that of the increase in phagocytic activity.

